African Crop Science Conference Proceedings Vol. 8. pp. 495-500
Printed in El-Minia, Egypt
ISSN 1023-070X/2007$ 4.00

Analysis of chlorophyll pigmentation for yield prediction in cowpea varieties (*Vigna unguiculata* (L) Walp)

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Abstract: Six cowpea genotypes; UCC-W, UCC-E, UCC-P1, UCC-P4, UCC-07 and UCC-V6 were evaluated in the research field (Technology village) of School of Agriculture, University of Cape Coast, Ghana during the 2006/2007 cropping season. The experimental layout was Randomize Complete Block Design with two replications. Each cowpea genotype leaflets were sampled at 5, 6, 7th weeks after planting for molar absorbtivity and optical density coefficients as indicators for yield prediction at seedling stage. Leaf chlorophyll content was extracted using spectrophotometer set at a wavelength of 634nm. The samples were irradiated using radio isotope directed towards the sample. There was significant genetic variability among the cowpea genotypes. High chlorophyll absorption and optical film density were obtained for UCC-W, UCC-07 and UCC-E with a corresponding high yield and yield components. Between 5th and 6th week after planting was efficient for yield prediction in the cowpea genotypes. Chlorophyll absorption and optical film density are positively and highly related, with high coefficient of determination for all the cowpea genotypes. Thus, either spectrophotometer or beta radiography or both could be used as yield prediction tool in screening and selection of cowpea genotypes.
Key words: beta radiography; chlorophyll; cowpea genotypes; regression spectrophotometer; yield components

Introduction

Legumes are important foodstuffs in the world especially in the tropical and subtropical regions. The most prominent in the leguminosae family is cowpea (*Vigna unguiculata* (L) Walp). They are second in importance only to cereals as source of protein. Cowpeas are rich in protein, iron, starch, calcium, phosphorus and Vitamins B, which make them excellent food even when eaten in small amount (Ebong, 1972; Frey, 1985; FAO, 1988). Apart from their nutritional value cowpeas are also of great importance in agriculture, where, they have the ability to fix atmospheric nitrogen in the soil for use by non-nitrogen fixing especially acidic soils and soils deficient in phosphorus, calcium and other nutrient (Purseglove, 1968).

In breeding programs where a number of new lines are tested against check varieties, an easy and early accurate assessment of yield potential can be an important tool for identifying promising genotypes and the ability to accurately predict yield of field crops such as cowpea allows breeders, producers, economic agencies, and buyers to make decisions with respect to crop production managements, pricing and available markets. The conventional method of determining yield and growth of many crops is time consuming, demand for huge financial, human and material resources to get the expected results. Many researchers had worked on conventional, morphological, yield simulations and climatological modeling for yield prediction (Ebong, 1972; Aryeetey, and Liang, 1973; Frey, 1985). However, it is important to develop a more effective approach to predict crop yield in cowpea without waiting till plant maturity or harvest period. Thus, a more recent yield prediction methods based on spectrophotometer and beta radiographic analysis of chlorophyll extract is relevant.

The use of spectrophotometer for automatic determination of absorbance of chlorophyll pigments as a function of the wavelength is exploited to determine the concentration of the pigment and relate it to the rate of growth and fruit production in cowpea. While, the relevance of the use of beta radiography as a screening method for plant growth capacity is in the assessment of the plant health status and yield (Lichtenthaler , 1990; Blackburn, 1998; Cerovic, *et al.,* 1999; Takeuchi, *et al.,* 2002; Anderson, 2003).

This study was, therefore, undertaken to assess genotypic variability in cowpea, determine the minimum developmental stage of cowpea at which yield can be predicted from the chlorophyll concentration, and to establish the basis for using chlorophyll pigmentation as molecular maker for screening and selection of cowpea genotypes.

Materials and Methods

Six cowpea genotypes; UCC-W, UCC-E, UCC-P1, UCC-P4, UCC-07 and UCC-V6 were cultivated in the research field (Technology village) of School of Agriculture, University of Cape Coast, Ghana during the 2006/2007 cropping season (April to July). All the genotypes were obtained from Crop Science Department, University of Cape Coast, Ghana. They are early maturing (60 to 75 days), semi-erect, white seeded except UCC-E that is light brown. Cape coast is a coastal area characterized by bimodal rainfall; the major season is usually from April to July with short dry spell in August. The minor rainy season occurs from September to November. Mean annual rainfall ranges from 900-1000mm. The annual minimum temperature is about 25.3. The relative humidity ranges between 80 – 90%. The soil types belong to the Benya soil series and the soil pH range between 5.8 and 6.5 (Asamoah, 1973).

Seeds of each cowpea genotype were sown in 10L perforated plastic buckets that were filled with equal amount of sandy loam soil. The soil was collected from a fallow site of the research farm situated at the technology village, School of Agriculture, University of Cape Coast, Ghana. The chemical composition of the soil were; organic carbon 0.56%, pH 6.3, phosphorus 64.02 ppm, potassium 0.57 Cmol kg, nitrogen 0.08% and calcium 3.68 Cmol kg. No fertilizer application was done because the soil was relatively fertile for cowpea cultivation. Weeds were uprooted from the perforated plastic buckets (pots) as soon as they appear to prevent competition between them and the cowpeas. The plants were watered uniformly once a week.

Four seeds were sown per pot and latter thinned to two seedlings per pot. Each genotype was planted in 5 pots thus 30 pots with a total of 60 plants. The experiment was replicated two times, thus, a total of 60 pots and 120 plants were used in this investigation. The pots were placed in an open space for uniform sunlight at equal distance. The experiment was arranged in a Randomized Complete Block Design (RCBD) with two replications.

Chlorophyll extraction

Leaf chlorophyll content was extracted using middle leaflets of the second and third leaf with reference to the terminal leaf of each genotype were picked each week at the 5th, 6th, and 7th week after planting. A 2mm diameter cork borer was used to take the sample disc from the leaves under investigation. Leaf tissue weighing 2g was crushed in a mortar and 80% acetone was added to it in sufficient quantities to allow the tissue to be thoroughly homogenized and then filtered off through a filter-funnel into a test tube. The chlorophyll extract was obtained for each of the genotypes (Ekanayake and Adeleke 1996)

Analysis of chlorophyll pigment

Spectrophotometer

The used spectrophotometer was a type 600 Jenway machine model 6100 which operate at 240v, 60w and at frequency of 50Hz. For its light source it uses a hydrogen light, type M/29 that operates at 6v and 10w. It has a wavelength range of 320-920nm and a bandwidth of 5nm. The spectrophotometer was set at wavelength of 634nm where, 5ml of chlorophyll extract was pipetted into a 30ml volumetric flask and the volume made up with 80% acetone. The thoroughly mixed chlorophyll extract was transferred into the corvette compartment of the spectrophotometer and the absorbance readings were taken.

Beta radiography exposure

The samples were radiographed by placing them on the x - ray film in a protective cover (light tight). The radio isotope was directed towards the sample on the film. The samples were irradiated for 30 minutes and the photographic film was developed with electronic processor, the film density of the radiography was then measured by a densitometer forming 0.001 density precision.

Data were recorded for yield components; number of peduncles per plant, Number of pods per plant, number of seeds per pod, pod weight (g) and 100 seed weight (g). The data were statistically analyzed with SAS for Microsoft Windows, Release 6.10 (SAS, 1989). Analysis of variance and comparisons between means were calculated. Relationships between absorbance and optical density of each cowpea genotypes were calculated using conventional regression and correlation approaches (SAS, 1989).

Results and Discussion

Mean square, mean yield components performance, leaf chlorophyll absorbance and optical density of six cowpea genotypes are presented in Table 1. Number of pods/plant, seeds/plant and leaf chlorophyll absorbance had significant effect among the genotypes while number of peduncle/plant, total pod weight, 100 seed weight and optical density were highly significantly affected among the genotypes. These implied that the cowpea genotypes evaluated express genetic or inherent variability, thus, it is possible to practice selection and subsequent crop improvement. The mean agronomic performance of the genotypes also confirms their differential performance as seen by the LSD 0.05. UCC-E had the highest number of peduncle/plant, number of pod/plant, total pod weight and 100 seed weight, which, was closely followed by UCC-W for all the traits except number of seed/plant which was highest TSS. UCC-V6 and UCC-P4 are the worst agronomic performers considering all the traits measured. Similar genotypic variability had been reported in cowpeas and other legumes (Ebong, 1972; Frey, 1985; Appiah, 1996; Afful, 2001).

Highly significant variations in leaf chlorophyll absorbance and optical density were observed among the cowpea genotypes (Table 1 and Fig. 1-6). UCC-W, UCC-07 and UCC-E are not significantly different in their molar absorbtivity coefficient as measured by spectrophotometer and optical density coefficient from beta radiograghy. Thereby, suggesting that their leaf chlorophyll content is photosynthetically similar in the production of photo assimilates and this reflected in the high yield and yield components obtained. UCC-P4 and UCC-06 had the highest absorbance coefficient but had the least optical density as measured by beta radiation, which was not reflected in their yield as they showed low productivity, and rather there was high vegetative growth as a result of partitioning of photosynthates. The difference in absorbance and optical density coefficients may be as a result of the phonological stages of the plant sampled and individual response to environmental conditions due to the difference in their genetic constituents in UCC-P4 and UCC-06. Thus, chlorophyll is influenced by both genetic and environmental factors which directly or indirectly influence yield. This result agrees with those of Lichtenthaler, *et al.*, (1986); Subhash and Mohanan, (1995); Blackburn, (1998); Takeuchi, *et al.*, (2002).

The relationship between absorbance of light by chlorophyll pigment and optical density of leaflets for each of the genotypes are presented in Fig.1-6 respectively. Positive and high linear relationships were obtained for all the studied genotypes. The coefficient of determination (R^2) here, measured the proportion of interaction accounted for by regression, the study revealed high coefficient of determination for absorbance versus optical density for all the genotypes. This implied that the use of either spectrophotometer or beta radiation could be used for yield and agronomic performance prediction before harvest at maturity or at seedling stage. This finding collaborated with those of Karlsson, (1992); Subhash *et al.*, (1993); Matsuzama and Komatsu, (2002).

Table 2 shows absorbance and optical density of the six cowpea genotype leaflets sampled at the 5th to 7th week after planting. There is significant difference between sampling cowpea leaflet for chlorophyll molar absorbance and optical density at 5th weeks after planting and 6th, and 7th weeks after planting but no significant difference between 6th and 7th weeks after planting for UCC-W, UCC-E, UCC-P1 and UCC-07. Thus, yield could be predicted as from 6th weeks after planting using either or both spectrophotometer and beta radiography. However, there was no significant difference between 5th and 6th weeks after planting for chlorophyll molar absorbtivity and optical density for UCC-P4 and UCC-V6. Thereby implying that yield prediction for these genotypes can be as early as the 5th weeks after planting. Early screening and selection of crop is important in plant breeding because a lot of time, human and material resources are saved during crop improvement programmes.

Table 1: Mean squares, yield component, leaflet absorbance and optical density of six cowpea genotypes

Variable/ Genotype	No. of Peduncle /Plant	No. of Pods /Plant	No. of Seeds /Plant	Total Pod weight (g)	100 Seed weight (g)	Absorbance (Spec.)	Optical Density (Beta Rad.)
Mean Square	8.46**	6.82*	7.29*	23.06**	10.13**	5.30*	5.56**
UCC-W	13.00	26	75.5	91	42	0.26	2.03
UCC-E	14.00	28	71.2	96	46	0.27	1.99
UCC-P1	11.00	15	60.4	63	34	0.28	2.01
UCC-P4	9.00	12	40.7	57	26	0.33	1.85
UCC-07	10.00	17	66.3	70.5	36	0.24	2.48
UCC-V6	8.30	7	38.9	46	24	0.33	1.94
LSD 0.05	2.03	5.72	10.28	15.11	8.43	0.06	1.09
CV (%)	9.4	7.6	13.1	16.7	4.4	8.7	10.2

*, ** significant at 5 and 1% probability level respectively

Table 2: Absorbance and optical density of six cowpea genotype leaves recorded from 5th to 7th growth phase

Absorbance (Spect.)								
Optical Density Genotype	5th Week	6th Week	7th Week	LSD 0.05	5th Week	6th Week	7th Week	LSD 0.05
UCC-W	0.15	0.22	0.29	0.08	2.65	2.89	3.01	0.16
UCC-E	0.24	0.29	0.32	0.03	2.18	2.39	2.41	0.05
UCC-P1	0.23	0.29	0.31	0.05	1.05	2.06	2.08	0.03
UCC-P4	0.20	0.22	0.23	0.03	1.99	2.14	2.20	0.23
UCC-07	0.18	0.23	0.27	0.04	1.30	2.07	2.15	0.10
UCC-V6	0.12	0.13	0.14	0.02	1.98	2.00	2.01	0.03

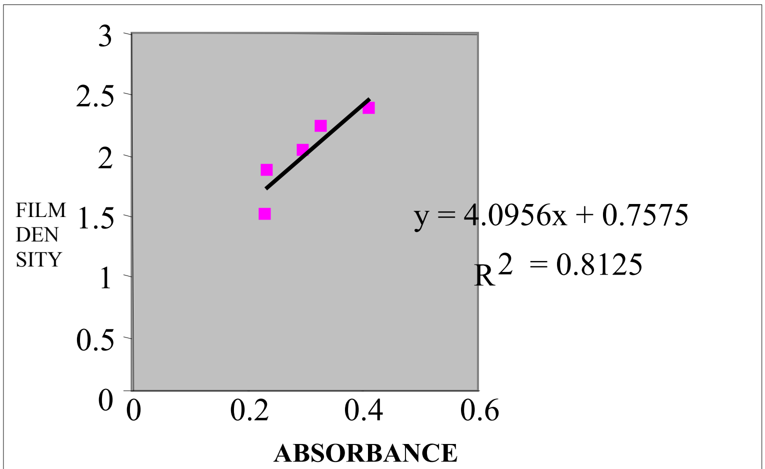

Fig.1: Relationship between light absorbance and optical density in UCC-W

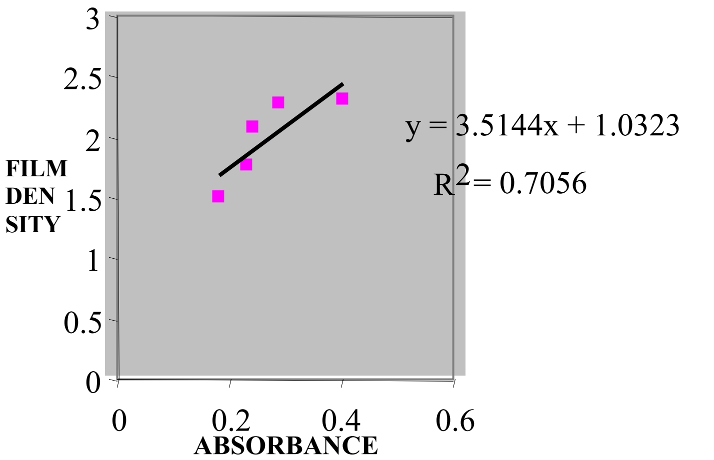

Fig.2: Relationship between light absorbance and optical density in UCC-E

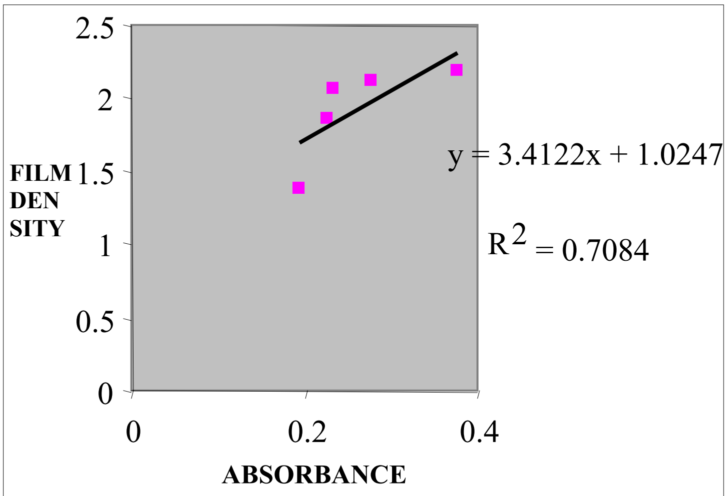

Fig.3: Relationship between light absorbance and optical density in UCC-P1

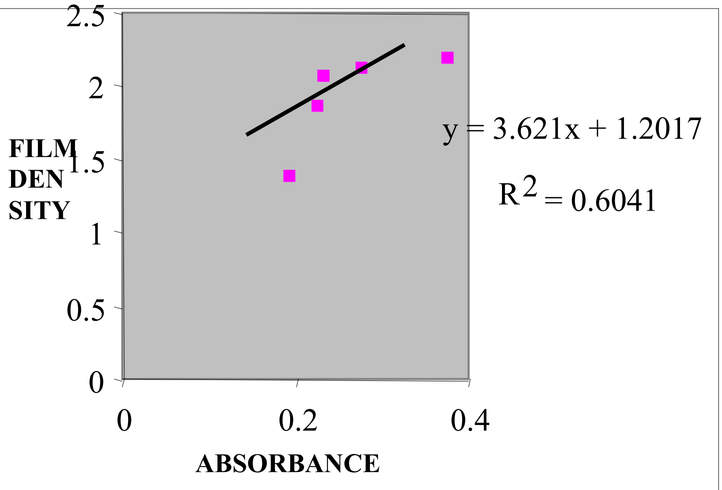

Fig.4: Relationship between light absorbance and optical density in UCC-P4

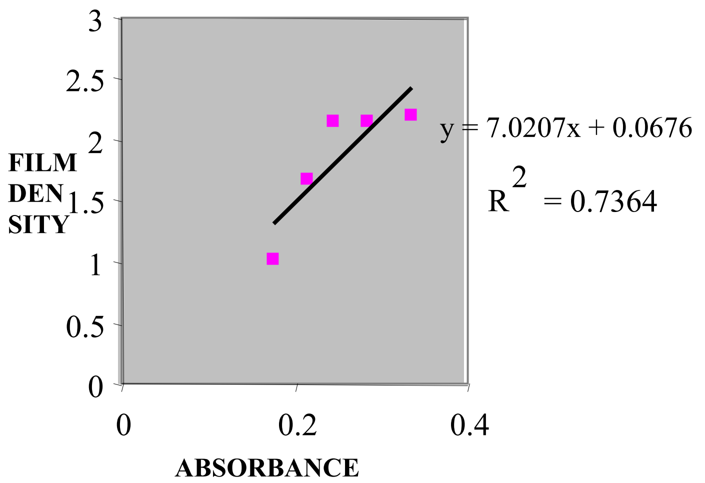

Fig.5: Relationship between light absorbance and optical density in UCC-07

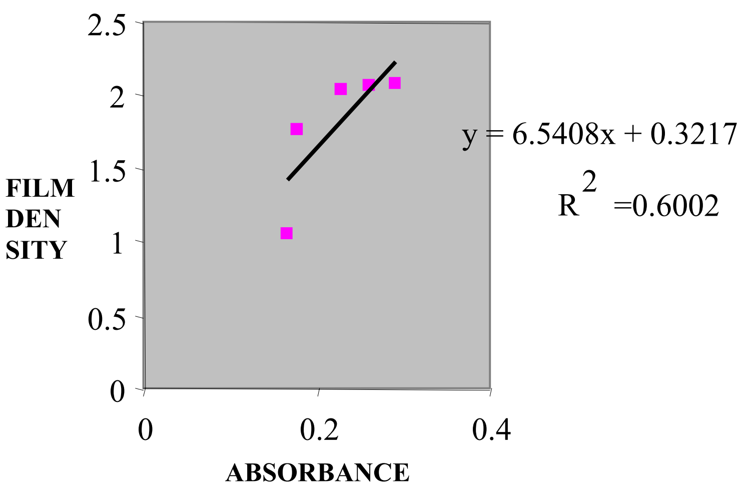

Fig.6: Relationship between light absorbance and optical density in UCC-V6

Acknowledgements

The authors are grateful to Alhaji Sulemana of Physics Department, University of Cape Coast, Ghana, Mr. Stevens and Mr. Patrick of radiography department of Central Region Hospital, Cape Coast, Ghana for their assistance in the course of this research.

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